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Insect Pupil Mechanisms

I. On the Pigment Migration in the Retinula Cells of Hymenoptera (Suborder Apocrita)

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Summary. The pupil mechanism of Hymenoptera (suborder Apocrita) has been studied by simultaneous recordings of transmission and reflection from the compound eye of virtually intact animals. It is confirmed that the light flux in the photoreceptors is controlled by pigment granules in the retinula cells; the pigment migration serves a pupil function. Experimental methods are described for investigation of the pupil process using only reflection measurements. Using polarised light, it is found that backscattered light from the rhabdom is more strongly depolarised than light backscattered from retinula cell pigment granules.

The dynamic characteristics of the pigment migration are determined more accurately than could be done previously with histological methods (Menzel, 1972a, b; Kolb and Autrum, 1972, 1974). The hymenopteran pupil mechanism has a familiar sigmoid intensity dependence; the time constant is 5–15 s. The pupil absorbance spectrum is broad, peaking at about 520 nm. The correspondence of this spectrum with known spectral sensitivities exemplifies that the pupil mechanism is a useful part of the visual system.

A. Introduction

Light dependent migration of pigment granules is a widespread phenomenon in insect eyes (see e.g., Exner, 1891; Mazokhin-Porshnyakov, 1969; Goldsmith, and Bernard, 1974). Apparently the function of the pigment granules is to control the light flux in the photoreceptors. Since pigment migration has been investigated almost exclusively with histological methods, little is known of dynamics and functional details (see Walcott, 1975). The main goal of our research will be to help to narrow this gap.

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As a starting point we recall that Kirschfeld and Franceschini (1969) demonstrated in the housefly *Musca domestica* the vivid pigment migration taking place in the retinula cells. In the dark small pigment granules are dispersed in the sense-cell soma and during the first few seconds of light adaptation they move towards the rhabdomere resulting in a decrease in transmission in the rhabdomeres and an increase in reflection from the eye. Franceschini (1972a, b; 1975), using the fruitfly *Drosophila melanogaster*, proved that the pigment migration is under direct control of activation of the visual pigment which is contained in the rhabdomeres; hence, the pigment migration system of flies acts as an automatic gain control mechanism analogous to the pupil mechanism of vertebrates.

With techniques related to those employed by Kirschfeld and Franceschini we have in the past studied the pupils of the blowfly and butterflies in relation to the photochemical properties of the visual pigment, and we have come to the conlusion that the function of these pupils is not merely the control of the intensity of the light but also of its spectral distribution (Stavenga et al., 1973, 1975; Stavenga, 1975b, c).

In the present paper we investigate the pupil mechanism of another important insect order, viz. the Hymenoptera (for preliminary accounts, see Stavenga, 1971, 1975a, b). Histological evidence for the view that the distribution of pigment granules in the visual sense cells of hymenopteran insects is a function of the state of light/dark adaptation, has been reported for ants (Menzel and Lange, 1971; Menzel, 1972a; Brunnert and Wehner, 1973) as well as for bees (Kolb and Autrum, 1972) (see Fig. 1).

In order to understand the action of migrating retinula cell pigment granules it is necessary to consider the fused rhabdoms of Hymenoptera as well as fly rhabdomeres as optical waveguides (De Vries, 1956; Kuiper, 1962; Varela and Wiitanen, 1970; Goldsmith and Bernard, 1974; Snyder, 1975; Snyder and Menzel, 1975; Stavenga, 1975a). An important property of optical waveguides is that the light flux is partially guided outside the boundary. This property is utilised in the pupil mechanism; pigment granules occurring near the rhabdom can frustrate the boundary wave by absorption and scattering. Thus by varying the number of granules near the rhabdom the transmitted light flux can be controlled. The transmission of the rhabdom is a monotonic function of the number of pigment granules near the rhabdom according to the theroretical approach set out by Snyder and Horridge (1972; see Snyder, 1975) for the essentially identical case of the cockroach rhabdom. In light-adapted retinula cells of ants the number of pigment granules near the rhabdom increases monotonically with the intensity of the adapting light (Menzel, 1972b). Furthermore, pigment migration in Hymenoptera is most probably controlled by visual pigment processes, as follows from selective adaptation experiments (Menzel, 1972b; Menzel and Knaut, 1973; Kolb and Autrum, 1974; cf. the case of the cockroach, Butler, 1972). Recalling the case of the fly we hence can state that several insect orders apply the same principle, namely automatic gain control by pigment granules in retinula cells.

The experimental results presented below provide more details of the pupil mechanism of Hymenoptera. First we shall describe the optical methods which

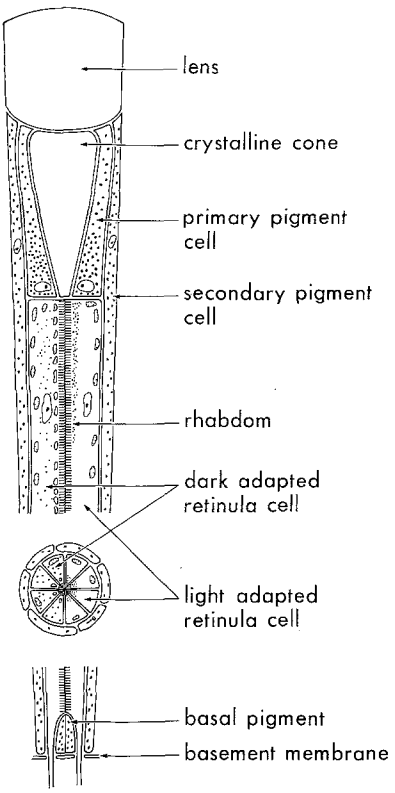

Fig. 1. An ommatidium of the hymenopteran compound eye is shown diagrammatically (redrawn from Varela and Wiitanen, 1970; Perrelet, 1970; Menzel, 1972a; Kolb and Autrum, 1972; Gribakin, 1975)

allow in vivo investigations (see Franceschini, 1975; Franceschini and Kirschfeld, 1976). We show that simultaneous measurement of transmission and reflection from the compound eye is a powerful tool to gain insight into the pupil mechanism. From the experimental results the dynamic and spectral properties of the pupil mechanism can be estimated.

B. Methods

The experimental techniques are essentially optical (see Stavenga et al., 1973; Stavenga, 1975b). The insect to be investigated is immobilized with wax and mounted on a goniometer (Fig. 2). The naked tip of a silvered and subsequently black painted quartz rod is inserted into the back of the insect's head through a small incision in the cuticle. The rod can function as a light guide to a photomultiplier, thus with normally incident illumination of the cornea it measures the orthodromic transmission (Kirschfeld and Franceschini, 1968) of the eye. The (orthodromic) reflection is measured via a half mirror and a microsoope to which a second photomultiplier is coupled. The microscope objective was a Luminar 40, the usual aperture 0.08. The illumination originates from 150 W Xe-lamps.

In the case of antidromic illumination (Kirschfeld and Franceschini, 1968) the quartz rod guides light into the back of the head. The light flux leaving the eye is measured then with the second photomultiplier. It is clear that orthodromic transmission and reflection can be recorded simultaneously whilst with antidromic illumination only the transmission is measured. Still the apparatus is versatile and instantaneous switching between the two modes of measurement is possible.

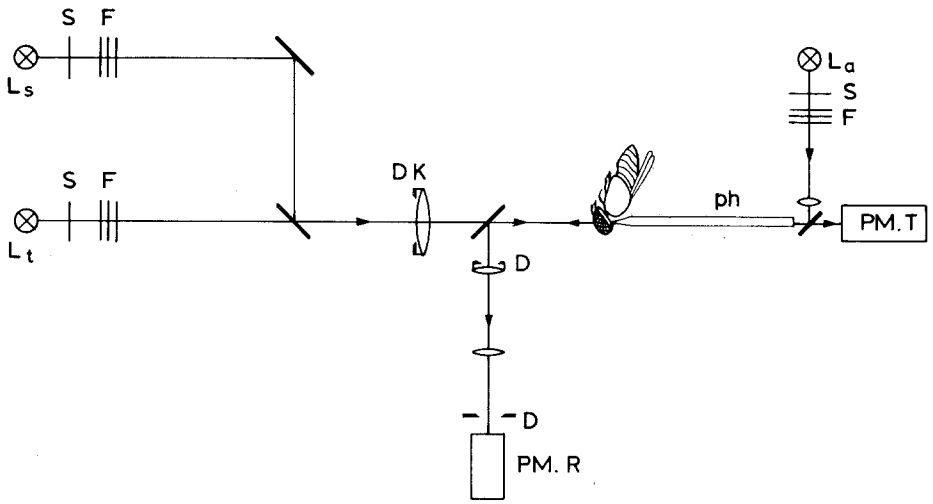

Fig. 2. The experimental set-up. Light delivered by two 150 W Xe-lamps L_s and L_t is focussed by the condenser *DK* on the compound eye of the insect. The transmitted light is guided by a quartz rod (photode) ph to a photomultiplier *PM.T.* The naked tip of the coated photode is inserted through a minor incision in the cuticle of the backside of the head. The orthodromic transmission of the ventral part of the compound eye is measured since in this eye region the cornea reflection of Hymenoptera is largely displaced from the pseudopupil. The reflection from the pseudopupil is measured via a microscope and the photomultiplier *PM.R.* With the latter photomultiplier the antidromic transmission also can be measured by guiding light, delivered by another 150 W Xe-lamp L_a, with the photode in the back of the head. Obvious accessories are adjustable diaphragms *D*, shutters *S*, neutral density and interference filters *F*. The photomultipliers type is EMI 6256 S

The reflection and antidromic transmission measurements are executed on the deep-pseudopupil (Franceschini and Kirschfeld, 1971b, 1976; Franceschini, 1972a, b, 1975). Details of the deep-pseudopupil are selectively measured by means of an iris diaphragm in front of the photomultiplier. Only the ventral eye regions are investigated, since here interference by corneal reflections can be satisfactorily avoided (see Exner, 1891).

The investigated Hymenoptera all belong to the suborder Apocrita.

C. Results

1. The Pupil Phenomenon

The properties of the hymenopteran pupil mechanism that may be determined using the methods described include: intensity dependence, time course of light and dark adaptation and the absorbance spectrum. The physical basis of the transmission and reflection recordings must obviously be understood clearly first. Initially, therefore, we must devote ourselves to an extensive analysis of the optical pupil phenomena. To begin with, the recordings of Figure 3 are discussed, so that the visual observations described in the next section may be the basis of a general interpretation of the occurring phenomena.

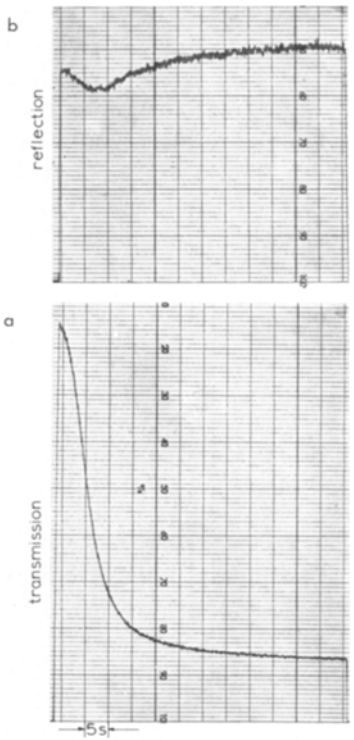

Fig. 3a and b. Simultaneously recorded orthodromic transmission **a** and reflection **b** from the wasp *Paravespula germanica*. An intense white illumination is applied during 1 min after a dark adaptation time also of 1 min. The transmission falls monotonically, probably as a result of the absorbing and scattering retinula cell pigment granules approaching the rhabdom until an equilibrium is reached. The reflection initially shows a decreasing phase parallel to the transmission course, but after about 10 s the reflection increases antiparallel to the transmission

Figure 3 shows the experimental transmission and reflection curves obtained from the wasp *Paravespula germanica*. The procedure was as follows. After a dark adaptation time $t_d = 1$ min, an intensive orthodromic illumination (white light, illumination time $t_l = 1$ min) induces a monotonic decrease in transmission of the eye (Fig. 3a). The simultaneously recorded reflection curve (Fig. 3b), however, has a biphasic shape. That dark adaptation after 1 min is not yet complete can be seen from Figure 8.

The recordings of Figure 3 resemble experimental curves obtained from flies (with the same methods; see Stavenga, 1975b). In view of the survey outlined in the introduction it is obvious to assume that the recorded transmission decrease (Fig. 3a) is caused by a monotonically increasing number of retinula cell pigment granules gathering near the rhabdom, where the granules attenuate the propagated light flux. The biphasic reflection curve, requiring a more complex interpretation, will be discussed after an account of some visual observations.

2. Visual Observations

Important properties of the pupil mechanism can be concluded from visual inspection as we shall describe now. Indispensable for a good comprehension of the following section are the basic studies of Kirschfeld and Franceschini devoted to the optics of insect eyes (Kirschfeld, 1967, 1969; Kirschfeld and Franceschini, 1968, 1969; Franceschini and Kirschfeld, 1971a, b, 1976; Frances-

chini, 1972a, b, 1975). The latter authors showed that antidromic light will leave the eye through the corneal facet lenses. With white antidromic illumination of a hymenopteran eye (e.g., of a wasp) the cornea-pseudopupil (which is observed with a microscope focused on the cornea) shows a reddish glow in a number of facet lenses, this number depending on the aperture of the microscope objective (see Kirschfeld, 1973). Focussing the microscope at the level where the ommatidial axes intersect one observes the deep-pseudopupil as a more or less circular reddish spot. This spot is the superimposed image of rhabdom tips: the antidromic light, before it leaves the eye is guided through the rhabdom.

Adding to the constant antidromic light an intense orthodromic illumination, causes the reddish spot gradually to diminish in intensity. In subsequent darkness the spot is restored (for recordings see Fig. 9). These effects occur in the order of seconds (Franceschini, 1975). So, even from simple visual inspection one can take for granted that it is the transmission of the rhabdom which diminishes upon light adaptation and recovers during dark adaptation.

That the origin of the transmission changes is indeed pigment migration can be seen from the (orthodromically) reflected light. Starting from the dark-adapted state one observes in the center of the deep-pseudopupil a reddish-purplish spot. This spot coincides perfectly in location and dimensions with the reddish spot which was observed with antidromic illumination (under exactly the same experimental conditions). Upon light adaptation this central spot diminishes in intensity and gradually a whitish annulus emerges. For excellent photographs of the latter phenomena, see Franceschini (1975, Fig. 16, or Franceschini and Kirschfeld, 1976, Fig. 7). An obvious interpretation of the reflection phenomena can now be forwarded.

The reddish-purplish reflection spot displayed in the dark-adapted state is light which is backscattered within the rhabdom (for the same phenomenon in flies, see Stavenga, 1975b). (We interpret the reddish-purplish colour in the Discussion). The backscattered light, travelling the reverse route in the rhabdom, has a second chance of being affected by the pupil granules. The influence of the pupil emerges in the first phase of the reflection curve (Fig. 3b), i.e., in the drop in intensity of the spot in the centre of the deep-pseudopupil. We note that the time course of this phase is similar to that of the transmission curve (Fig. 3a). The annulus arising with light adaptation around the rhabdom image is light backscattered from the retinula cell pigment granules surrounding the distal end of the rhabdom (Franceschini, 1975). The intensity of this backscattered light increases during adaptation, and this reflection increase overshadows the initial reflection decrease (Fig. 3b). When the reflection from the pigment granules is quite intense, as holds for some bumblebees, the second phase prevails completely (see Fig. 7). A few experiments to check our interpretations are described next.

3. The Two Components of the Reflection Deep-Pseudopupil

We claim above that the central spot in the deep-pseudopupil is light backscattered from the rhabdom and that the annulus is light backscattered from

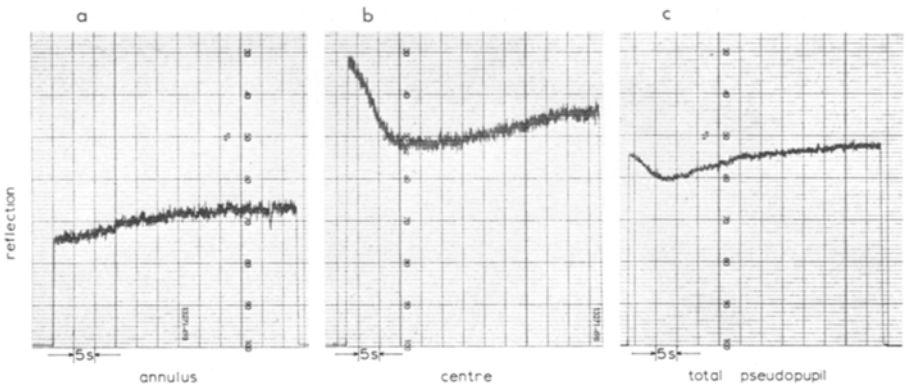

Fig. 4a–c. Reflection from different parts of the deep-pseudopupil during light adaptation. In Hymenoptera the deep-pseudopupil displays in the dark-adapted state a reddish-purplish centre, which vanishes upon light adaptation and then becomes surrounded by a whitish annulus. **a** The time course of the emergence of the annulus. **b** The course of the vanishing of the central reflection is the first phase. **c** The summed total of both courses, being the reflection of the total deep-pseudopupil. The number of retinula cell pigment granules near the rhabdom increases during light adaptation. Hence the intensity of directly backscattered light by the granules increases, thus inducing the monotonic reflection increase from the annulus. On the other hand the absorbing properties of the granules cause the backscattering of light within the rhabdom to fall, resulting in a pronounced reflection decrease from the centre. The reddisch-purplish coloured backscattered light from the rhabdom probably originates from self absorption by the visual pigments

pigment granules gathering near the rhabdom. By measuring selectively the reflection from either the centre or the annulus this view is strengthened, as shown by Figure 4 (same wasp as in Fig. 3). The illumination is white, illumination time $t_l = 1$ min; the time in darkness previous to the illumination $t_d =$ 1 min.

Figure 4a shows the time course of the reflection from a part of the annulus only; we note that this reflection increases monotonically. Screening off all but the centre of the deep-pseudopupil yields a reflection time course (Fig. 4b) which initially strongly resembles the transmission course (Fig. 3a). Subsequently however, there is a slight increase in reflection which must be attributed to light scattered by pigment granules leaving the rhabdom through its distal end. [Remember, the deep-pseudopupil centre is the image of the rhabdom tip]. Comparison of Figure 4a and Figure 4b with Figure 4c, which represents the reflection from the centre plus the complete annulus, shows an evident dichotomy in the two reflection components. We show next that the two components can also be usefully separated by illuminating with polarised light.

4. The Use of Polarised Light

From our work on the fly (Stavenga, 1975b) we know that backscattered light from retinula cell pigment granules can be selectively obstructed by using crossed polarizers. Figure 5 shows recordings with polarised light. Apart from a polarizer,

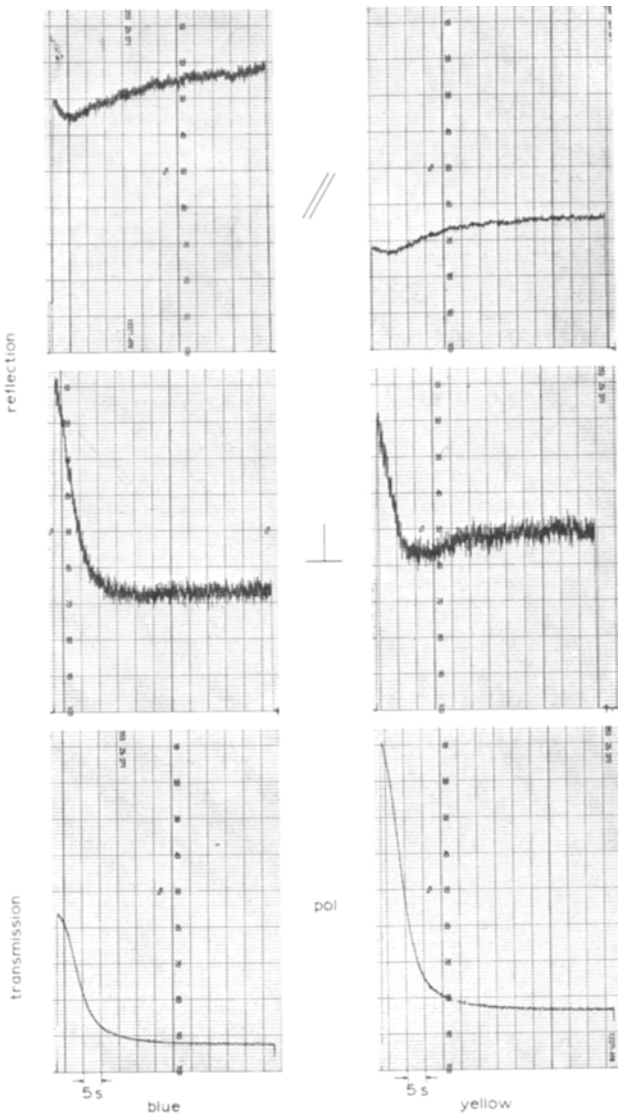

Fig. 5. Light adaptation with polarized light. A broad-band blue or alternately yellow filter was also placed in the illumination beam. The lower curves show the orthodromic transmission during 1 min of light adaptation after 1 min dark adaptation. The analyser in front of the reflection photomultiplier was either parallel (upper curves) or crossed (middle curves) to the polarizer. With parallel polaroids a biphasic reflection time course results from the total pseudopupil similar to that obtained with illumination by unpolarized light (Figs. 3b, 4c). On the other hand, crossed polaroids strongly depress the second phase. As the first phase represents the backscattering from the rhabdom and the second phase is caused by backscattering from retinula cell pigment granules the degree of polarization of the granule backscattering proves to be much less changed than that of the rhabdom backscattering

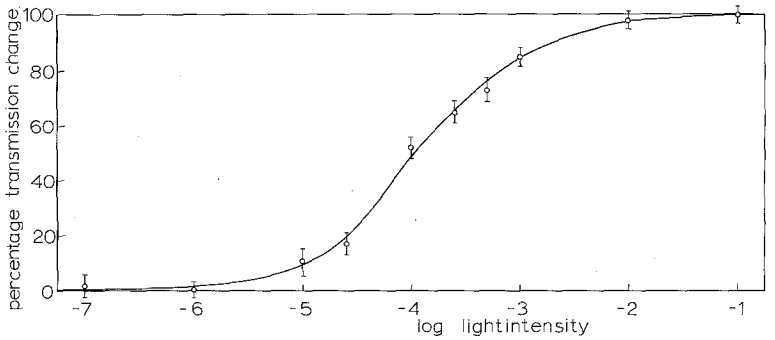

Fig. 6. Intensity dependence of the pupil. The effective range of the pupil mechanism covers a few log units (bumblebee *Bombus lapidarius*)

a broad-band blue filter or a broad-band yellow filter is placed in the illumination beam. An analyser is put in front of the reflection photomultiplier. The time of light and darkness again is $t_l = t_d = 1$ min (same wasp as in Figs. 3 and 4). In the lower traces the transmission curves are shown and are clearly similar to the transmission curve of Figure 3a. Furthermore the reflection curves obtained from the whole deep-pseudopupil with parallel polarizers (upper traces) are similar to those in Figure 3b. However, the middle traces recorded with crossed polarizers show a greatly depressed second reflection phase. The interpretation of this effect is quite identical to that given in the case of the fly (Stavenga, 1975b). Light which has been propagated in the anisotropic rhabdom will suffer much more depolarization than light backscattered by the pigment granules (which are distally located in the visual sense cells).

Having analyzed so far the components of the optical phenomena we now turn to some characteristics of the pupil mechanism.

5. Intensity Dependence of the Pupil Mechanism

The first characteristic of the pupil mechanism we look at is its intensity dependence. The pupil controls the transmission of light in the rhabdom, so the state of the pupil in an equilibrium, established by adapting lights of various intensity, is expressed by the change in transmission with respect to the transmission in the dark-adapted state. Figure 6 gives the intensity dependence of pupil equilibria in the case of the bumblebee *Bombus lapidarius*. A familiar sigmoid relation proves to exist between the percentage decrease in transmission and the logarithm of the illumination intensity.

6. The Time Course of Light- and Dark-Adaptation Processes

With orthodromic illumination the effect of the migrating pigment granules can only be observed during light adaptation. Still the pupil time course during

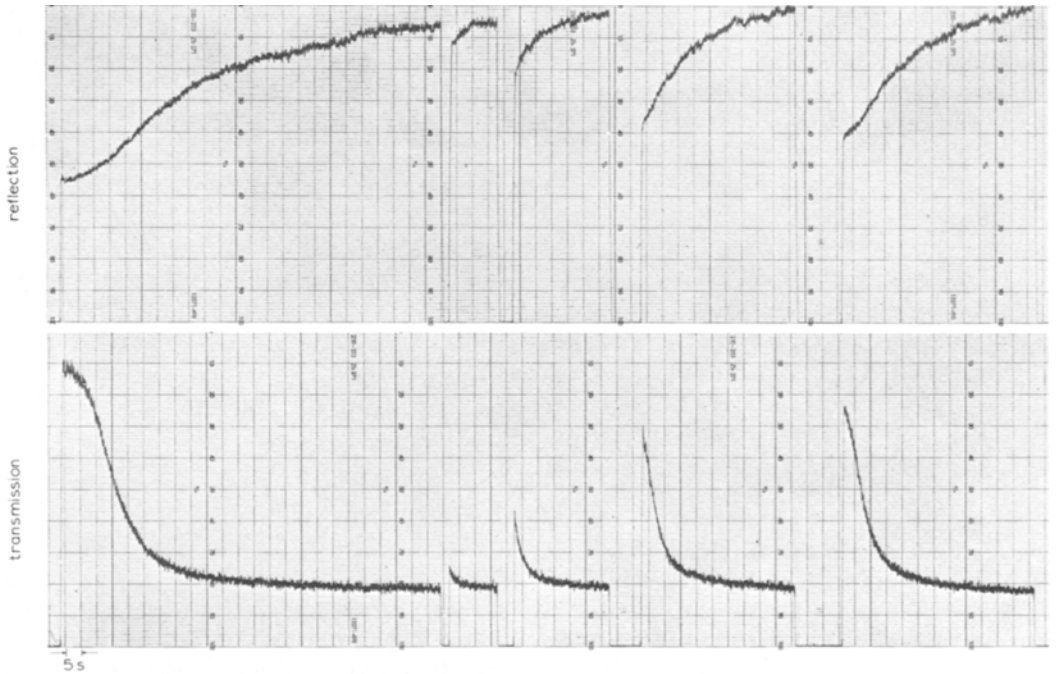

Fig. 7. Simultaneous recordings of transmission and reflection (bumblebee *Bombus pascuorum*). Illumination was applied after 1 min dark adaptation and subsequently dark times of respectively 2.5, 5, 10 and 15s were intercalated. The pupil closes during illumination and reopens in the dark with a similar time constant of about 10–15 s. In the case shown here the reflection increase due to pigment granule backscattering is so pronounced that the first phase emerging in previous figures is overridden by the second phase. Transmission and reflection differ in their functional dependence on time

dark adaptation can be extracted from measurements during orthodromic illumination after varying times in the dark. As an experimental example Figure 7 presents the orthodromic transmission and reflection time course after respectively t_d = 60, 2.5, 5, 10 and 15 s (in the bumblebee *Bombus pascuorum*). It proves that in darkness both transmission and reflection recover quickly; the dark adapted values are attained in about 1 min. Figure 7 furthermore shows that the increase in reflection proceeds more sluggishly than the decrease in transmission (see also Fig. 3, 4, 5).

Longer times in darkness have a strong delaying effect on the subsequent light adaptation time courses. This influence is investigated in the experiment of Figure 8 (*Paravespula germanica*). The intensity and time of illumination have been constant with t_l = 1.5 min, whilst the time in darkness, t_d, is varied. In Fig. 8a the transmission curves, following the time in darkness, t_d, indicated, are drawn superimposed. Apparently, the longer the previous darkness time the larger the initial transmission value and the slower the light adapted state is reached. An obvious interpretation of these facts is that the number of pigment

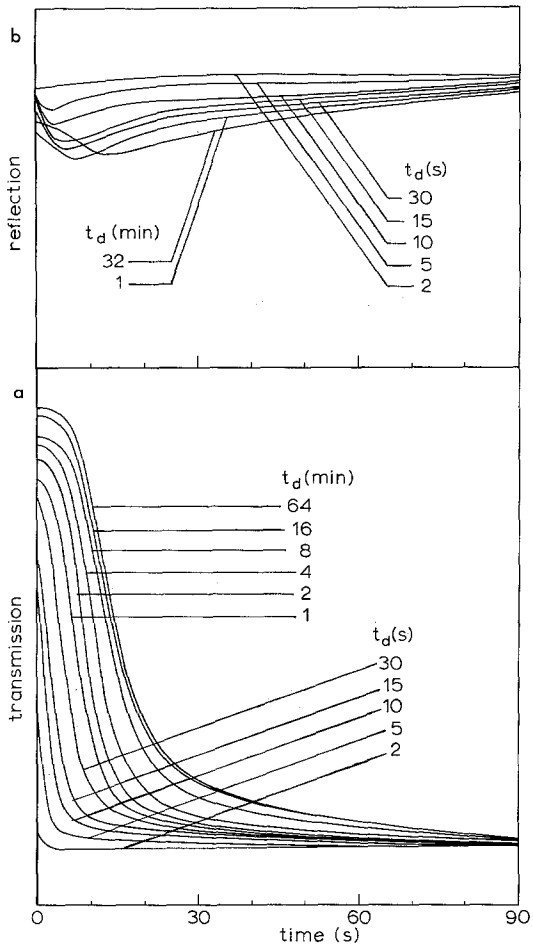

Fig. 8. Transmission and reflection time course as a function of the foregoing dark adaptation time t_d. The longer t_d is, the more sluggishly the pupil closes. (Some recordings of the series have been omitted for clarity's sake)

granules near the rhabdom progressively falls with longer t_d, so causing an increased initial transmission value. The main departure of pigment granules from the sphere of influence of the rhabdom will occur in the first minute after illumination. In the following minutes the migration away from the rhabdom slowly continues. Therefore, after longer times in darkness light adaptation will proceed more slowly since the pigment granules have to cover longer distances.

The effect of the time in darkness on the reflection curves is shown in Figure 8b. Here also the time course is slowed down with longer t_d, the magnitude of the effect being different for the two phases. (For clarity's sake a number of curves from the series have been omitted in Fig. 8.)

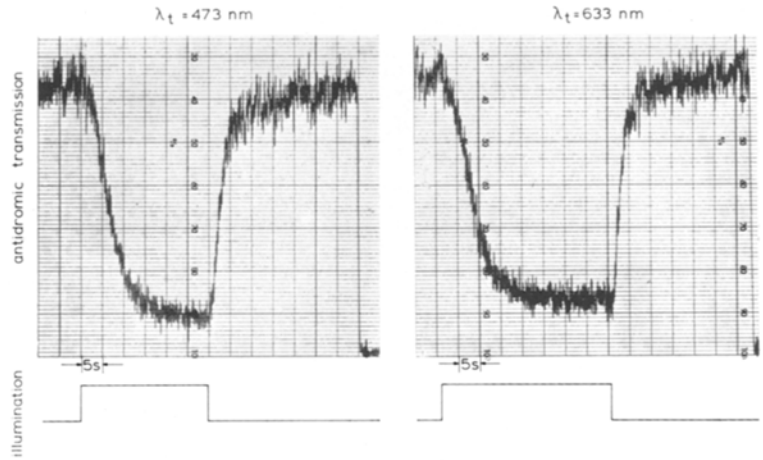

Fig. 9. Antidromic transmission during light and dark adaptation at test wavelengths $\lambda_t = 473$ nm and 633 nm respectively induced by orthodromic illumination

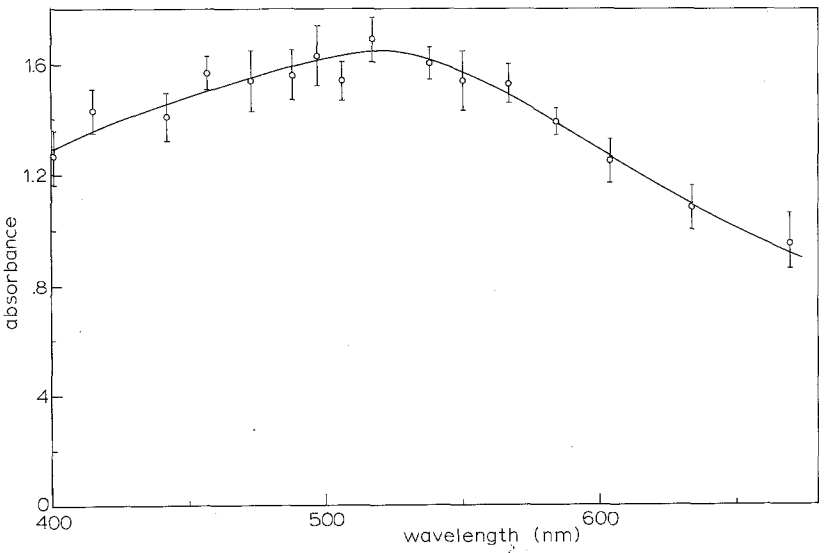

Fig. 10. Absorbance spectrum of the pupil of the wasp *Paravespula germanica* calculated from the extreme changes in antidromic transmission induced by saturating orthodromic light

A direct method to record the dark adaptation course is the application of antidromic transmission. Examples are given in Figure 9 showing the antidromic transmission course at test wavelengths $\lambda_t = 473$ nm and 633 nm respectively; light adaptation is induced by orthodromic illumination of 30 s duration. The time course of both light- and dark-adaptation is similar, the time constants being 5–10 s.

7. Pupil Absorbance Spectrum

The transmission changes as recorded in Figure 9 are well suited to yield the absorbance spectrum of the pupil. In the wasp (*Paravespula germanica*) we have measured the antidromic transmission in the dark-adapted state and the saturated light-adapted state at various wavelengths in the visual range. The napierian logarithm of the ratio of both transmission values yields the pupil absorbance spectrum (Fig. 10). The spectrum peaks at about 520 nm and covers a large wavelength range. The screening function apparent from Fig. 10 will be discussed below.

D. Discussion

1. The Pupil Mechanism: Light Control by Retinula Cell Pigment Granules

Pigment migration correlated to light-dark-adaptation in retinula cells of the compound eyes of Hymenoptera has recently been demonstrated with histological methods (Menzel and Lange, 1971; Kolb and Autrum, 1972). It has been proposed that the pigment migration serves a pupil function; the light flux in the photoreceptors is controlled by the amount of pigment granules near the boundary of the rhabdom. Convincing evidence for this view is provided by our optical measurements on living and virtually intact animals. Both visual observations and photometric measurements show that during light adaptation the transmission of the rhabdom decreases and during dark adaptation the transmission increases (Figs. 3a, 7–9). These processes are directly correlated to, respectively, the appearance or vanishing of retinula cell pigment granules near the rhabdom as follows from reflection measurements (Figs. 3b, 7, 9, and Franceschini, 1975; Franceschini and Kirschfeld, 1976).

We conclude that a fall in transmission induced by illumination indicates control of light flux by pigment granules. A valuable experimental result from our analysis of the reflection phenomena is that transmission-like curves can be obtained in reflection either by measuring only from the centre of the deep-pseudopupil (Fig. 4) or by applying polarised light (Fig. 5). Since the transmission is the more physiologically interesting quantity this experimental outcome can be exploited in the case of animals where transmission measurements are hampered. Actually we performed simultaneous measurements of transmission and reflection in the wasp, bee and bumblebee whilst in ants no successful transmission measurements could be made. Since the recorded transmission and reflection time courses are always essentially similar we assume that the stated conclusions hold for hymenopteran eyes generally. Related reflection phenomena to those reported in this paper can be observed in the compound eyes of other insects with fused rhabdoms, e.g. locust (Franceschini, 1975), dragonfly, cricket and cockroach (see Stavenga, 1972). The understanding of the details of the reflection deep-pseudopupil described above hence may be of value to the study of a wide variety of insects, especially those in which transmission measurements are difficult. A further discussion on optics hence will be worthwhile.

2. The Central Spot of the Deep-Pseudopupil

In this section we interpret the colours displayed by the central spot of the deep-pseudopupil. This spot is the superimposed image of rhabdom tips. Applying antidromic white light the central spot is coloured reddish (Section C.2). The antidromic light passes, in sequence, the transparent visual ganglia, basal pigment, is transmitted through the rhabdom and leaves the rhabdom through its distal end. Finally the light travels through the transparent cone and lens before being observed. Hence the only substantially absorbing substances in the lightpath are the basal pigments and the visual pigments in the rhabdom. The most appropriate candidate for the observed red-filtering is the basal pigment which according to Gribakin (1975, Fig. 8.5) is found in the expanded processes of the cone cells forming an absorbing layer at the end of the rhabdom. This view fits in with known absorbance spectra of hymenopteran screening pigment (see below, Section D. 4). (The basal red filter also is present in the eye of *Drosophila*; see Franceschini, 1975; Franceschini and Kirschfeld, 1976). We remark here that the basal pigment in fact is a substantial draw-back for accurate transmission experiments. More specifically, reliable measurements of orthodromic transmission at the intensity threshold of the pupil mechanism are strongly hampered owing to the signal drop caused by the basal pigment. Since with antidromic illumination the light filter stands in front of the photoreceptor reliable sub-threshold measurements in this case can be executed (see Fig. 9).

With orthodromic illumination of the ventral part of the dark-adapted eye the deep-pseudopupil displays a purplish-coloured central spot when observed in reflection (Section C.2). The spot in this case is light backscattered from the rhabdom. Preliminary measurements yield that the backscattering of the honeybee rhabdom is minimal at about 530 nm. In the ventral region of the honeybee eye green absorbing receptors are most abundantly demonstrated (Autrum and von Zwehl, 1964; Gribakin, 1972, 1975; cf. ant, Menzel, 1972b; bumblebee, Vishnevskaya and Mazokhin-Porshnyakov, 1972). Hence a suggestive interpretation of the reddish-purplish colour of the rhabdom image is that it originates from self-absorption by the visual pigments in the rhabdom. Furthermore, we have observed in both transmission and reflection photochemical effects (unpublished), very reminiscent to those detected in butterflies (Stavenga, 1975c), which pointed to a green absorbing rhodopsin. Again, these observations may be of value to the study of visual pigments in vivo in insects where transmission measurements are difficult.

3. Intensity Dependence and Time Course

We discuss in this section the relation between both the distribution of pigment granules in the retinula cell and their effect on the light flux, as a function of the illumination intensity and time. Firstly, in equilibria established by various adapting light intensities the pupil state is characterized by the percentage change in transmission (Fig. 6). The hymenopteran pupil has a monotonic, sigmoid

dependence on light intensity. A similar monotonic, sigmoid curve was obtained by Menzel (1972b) for the dependence of pigment movement on intensity by counting the number of pigment granules near the rhabdom in EM-sections. We hence may conclude that the transmission of the rhabdom T_r is a monotonic function of the number of pigment granules n_g near its boundary. Presumably the function T_r (n_g) is exponential (see Snyder and Horridge, 1972; or Snyder, 1975).

The time course of the pupil during light adaptation shows a monotonic decrease of the transmission, which obviously results from a monotonic increase in the amount of absorbing and scattering pigment granules near the rhabdom. A concomittant monotonic increase in reflection was measured in the deep-pseudopupil from the annulus. Hence also the reflection of the annulus R_a is a monotonic function of the number of the pigment granules near the rhabdom. Clearly the functions $R_a(n_g)$ and $T_r(n_g)$ are dissimilar as follows from inspection of the relevant figures (Figs. 3, 4b). The reflection of the centre of the deep-pseudopupil, being backscattering from the rhabdoms, R_r is also dependent on n_g as follows from Figures 4 and 5. Since $R_r(n_g)$ behaves like $T_r(n_g)$, it will approximate an exponential function. On the other hand $R_a(n_g)$, the annulus reflection, probably more or less approaches a linear function. The sum of R_r and R_a which is the reflection of the total pseudopupil therefore can take a biphasic shape. Having so far explained the reflection curves in terms of a change in pigment distribution it is an obvious statement that a more quantitative analysis of the indicated functions will give more detailed insight in the intracellular processes.

A substantial change in the pigment distribution occurs after longer times of darkness as follows from the subsequent light adaptation time course (Fig. 8). Similar strong delaying effects caused by long dark adaptation are detectable in butterflies (Stavenga, 1975c; Stavenga et al., 1977) and flies (Stavenga, Witpaard and Kuiper, in preparation). The experimental curves can be interpreted on the basis of the histological observations of Menzel and Lange (1971) and Brunnert and Wehner (1973) showing that in the retinula cells of ants the pigment granules migrate both radially and longitudinally. This interpretation will be forwarded in a future account of experiments on the pupil mechanism of blowflies.

4. Pupil Absorbance Spectrum

The pigment granules absorb and scatter light. The first of these effects is most important for the light control action as we argue below. Yet we must mention a third possibility for controlling the light flux in photoreceptors, namely, varying the refractive index of the surrounding medium. As mentioned before the fused rhabdom functions as an optical waveguide and the transmitted light flux critically depends on the difference in refractive index of rhabdom medium and surroundings. A tight envelope of pigment granules hence in principle can change this difference substantially. At present it is difficult to evaluate both this possibility and also the contribution of scattering to the measured

absorbance changes. However, our experimentally determined pupil absorbance spectrum appears to conform in shape with the broad absorbance spectra, peaking at about 500–550 nm, obtained for hymenopteran screening pigment granules in fixed eye slices and in fresh squash preparates (wasp, Höglund et al., 1970; honeybee, Strother and Casella, 1972; see Langer, 1975). We therefore assume that waveguide and scattering effects are of secondary importance and that the absorption properties of the pigment granules determine the main part of the pupil spectrum. This conjecture is in line with Langer's (1975), namely that the receptor cell granules have similar extinction properties as those of the primary pigment cells.

Comparing the visual sensitivity spectra of the wasp (ERG, Menzel, 1971) with its pupil absorbance spectrum (Fig. 10) we see close coincidence of peak wavelengths. Furthermore from the broad pupil spectrum it may be concluded that the pupil is well-suited to perform a protection function at least for the green receptors (see Langer, 1975). Evidently the visual cell pigment granules in Hymenoptera, as in other insects (see Introduction) must prevent the visual cells from overstimulation and thus indeed act together as an effective pupil.

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